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Fig. 1

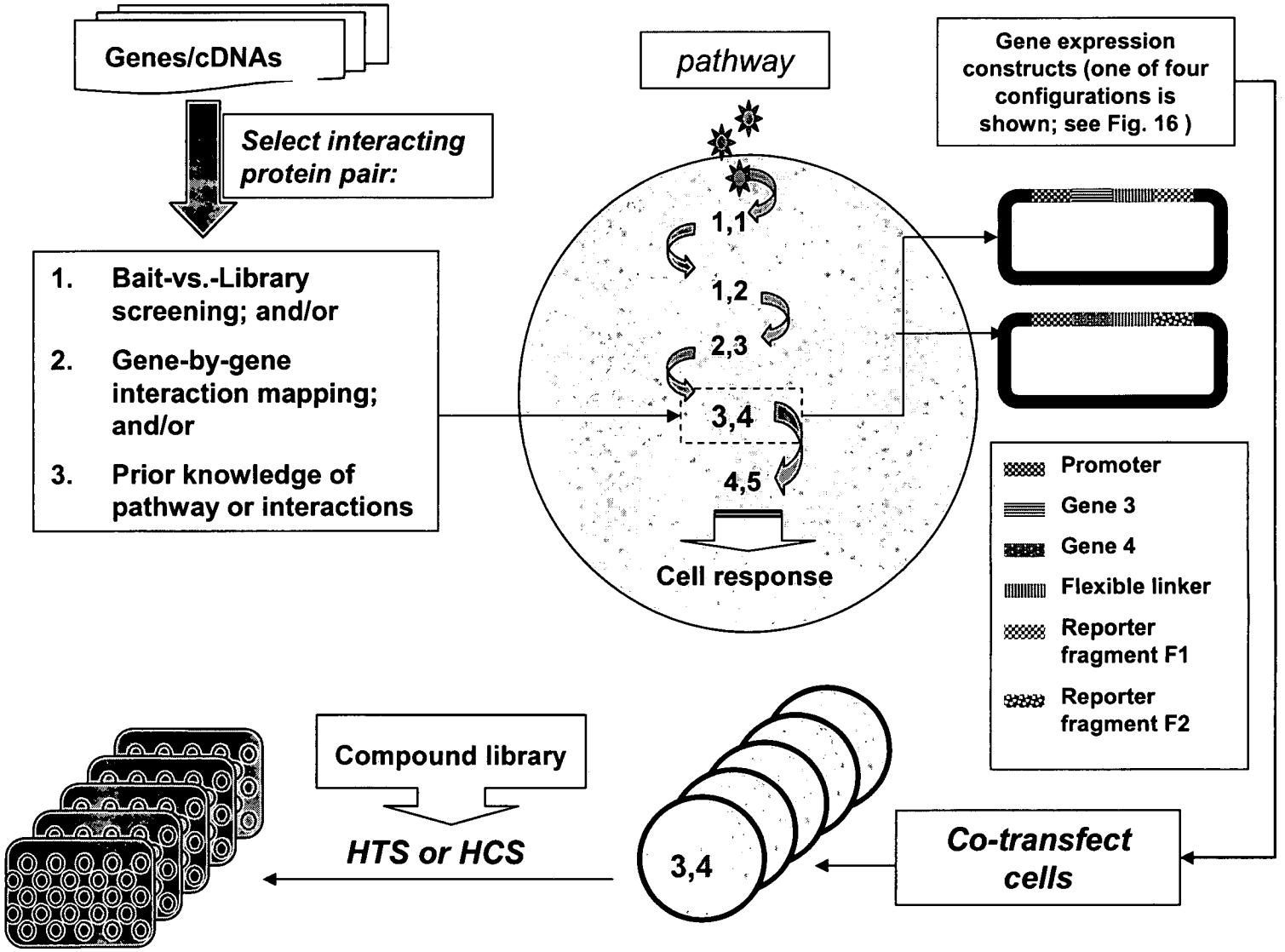
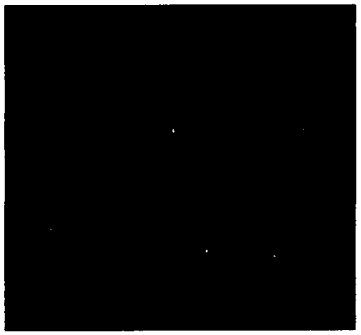
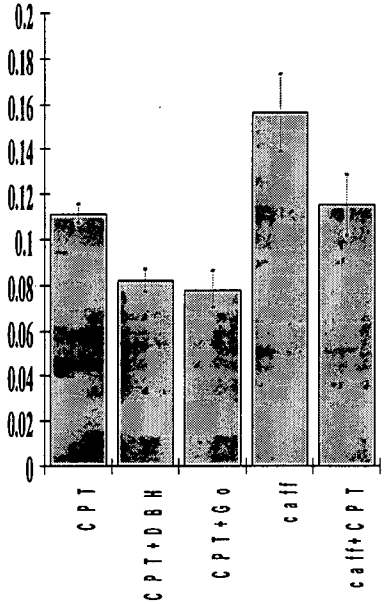

Genes/cDNAs
Select interacting protein pair:
1. Bait-vs.-Library screening; and/or
2. Gene-by-gene interaction mapping; and/or
3. Prior knowledge of pathway or interactions
pathway
1,1
1,2
2,3
3,4
4,5
Cell response
Gene expression constructs (one of four configurations is shown; see Fig. 16)
Promoter
Gene 3
Gene 4
Flexible linker
Reporter fragment F1
Reporter fragment F2
Co-transfect cells
3,4
Compound library
HTS or HCS

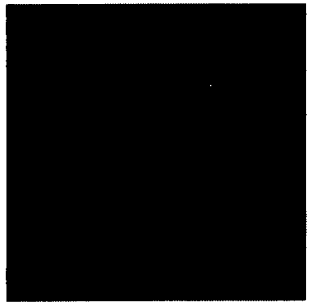
Fig. 2 Assays for the DNA damage response pathway

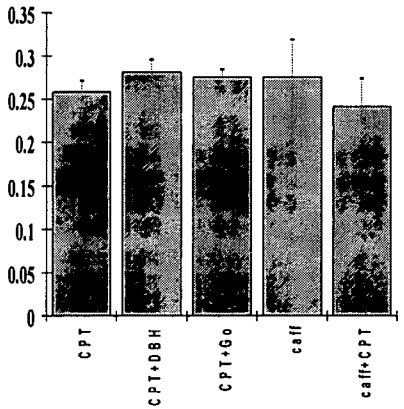
p53/ Chk1





p53/ p53





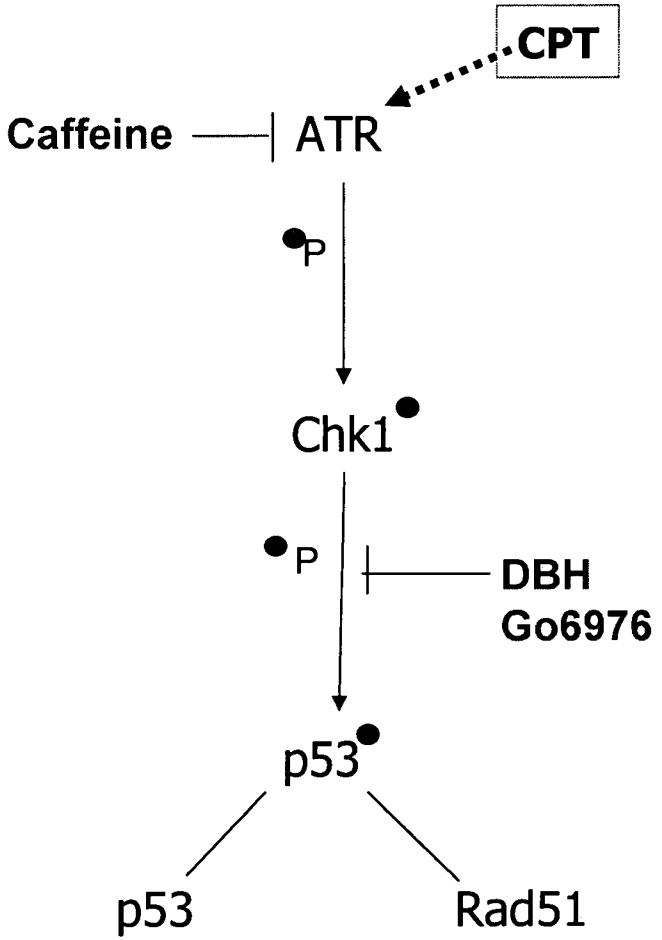
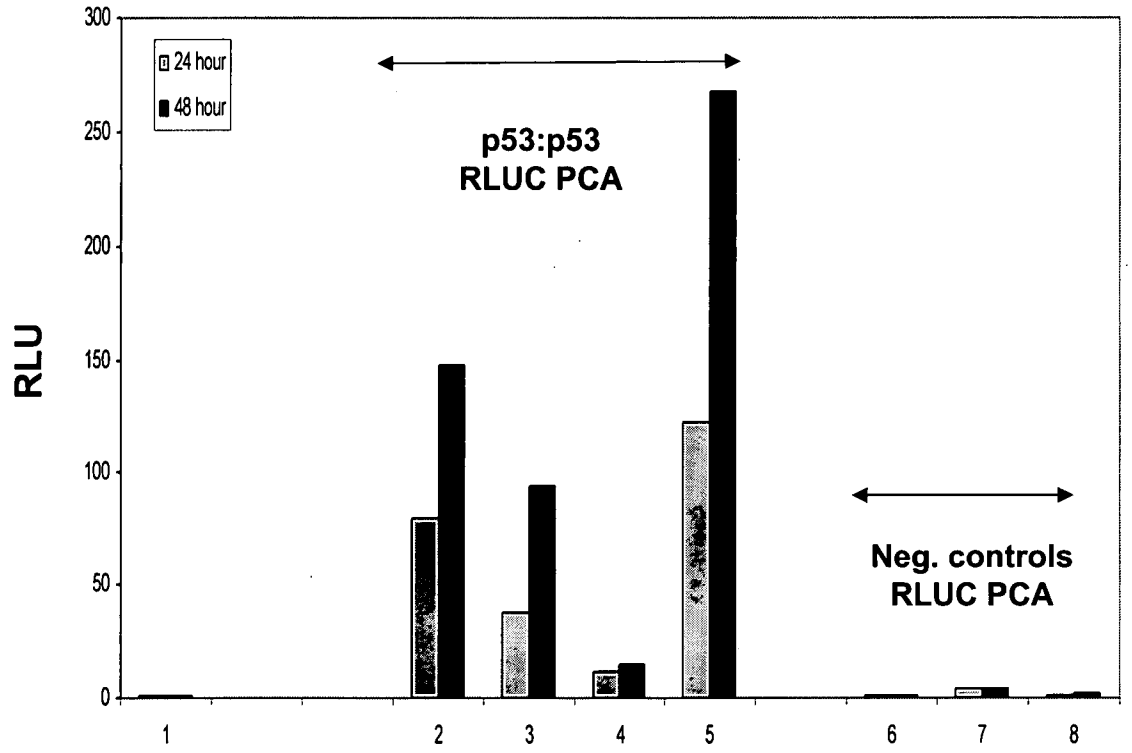


Fig. 3(A) A Luminescent PCA (RLuc PCA) for high-throughput screening (HTS)



Legend

1:background (substrate)
2: p53-RL[1]: p53-RL[2]
3: p53-RL[1]: RL[2]-p53
4: RL[1]-p53: p53-RL[2]
5: RL[1]-p53: RL[2]-p53
6: Pdk2-RL[1]: STAT1-RL[2]
7: RL[1]-STAT1: Pdk2-RL[2]
8: RL[1]-Pdk2: STAT1-RL[2]

Fig. 3(B) Effect of camptothecin (CPT) treatment on p53/p53 (Renilla luciferase PCA)

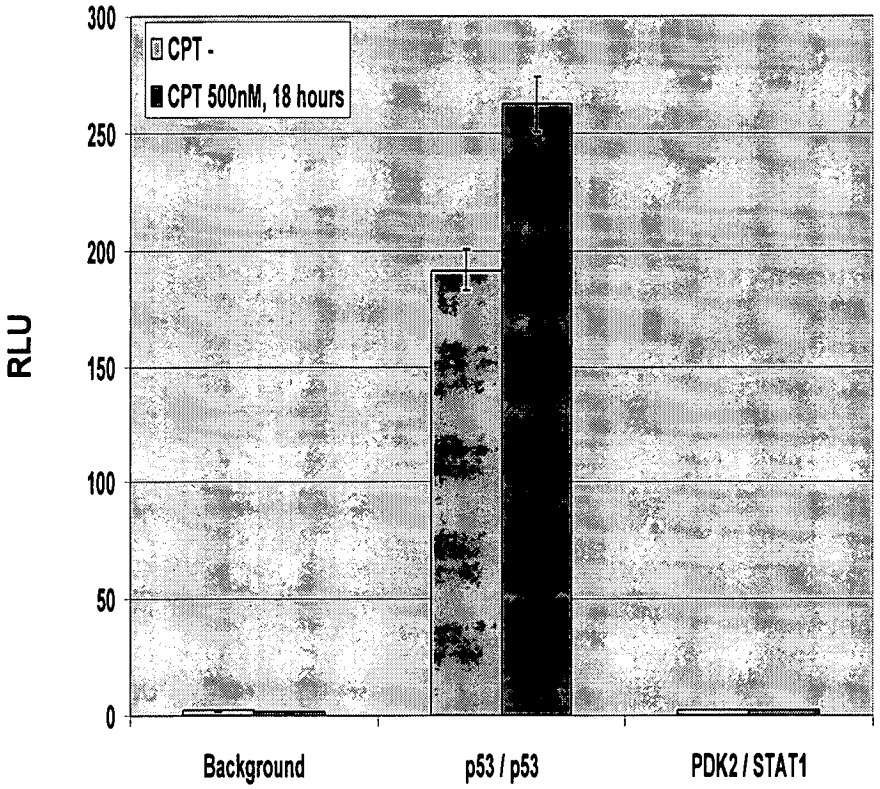
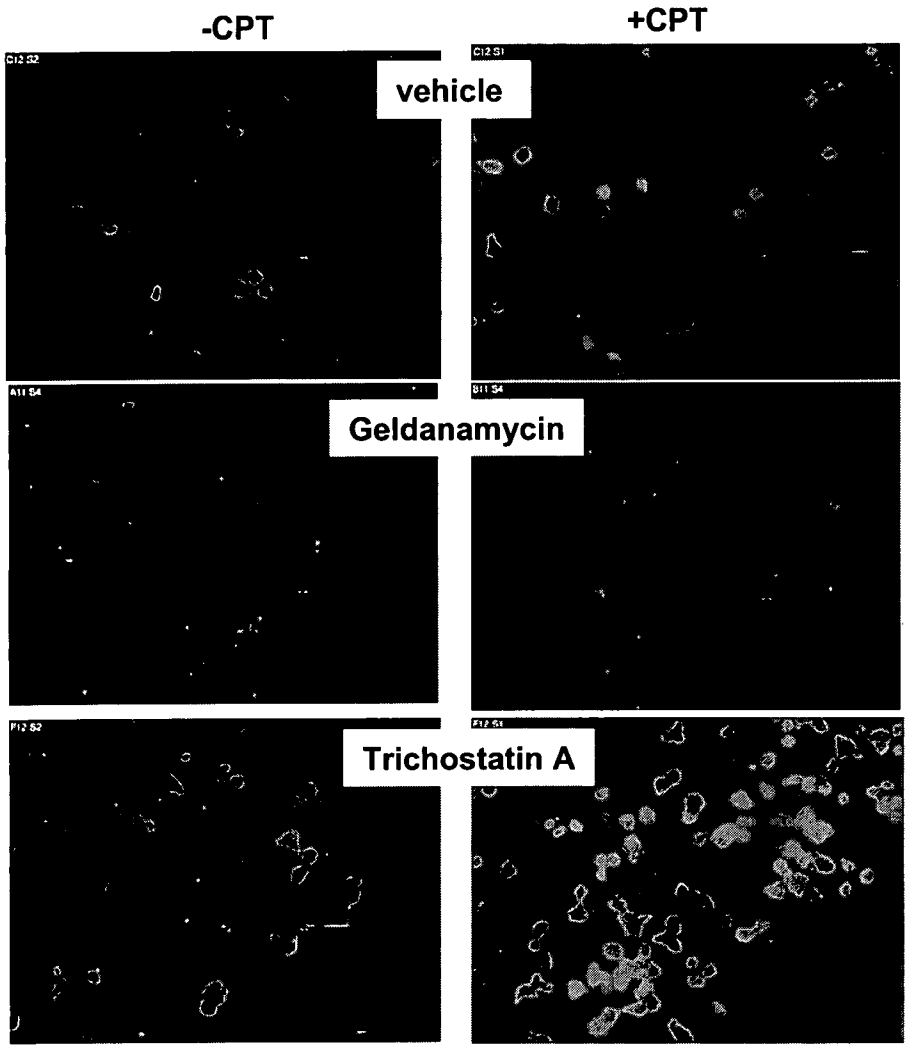


Fig. 4 IFP PCA demonstrating effects of drugs on p53/p53 in the presence and absence of camptothecin (CPT)



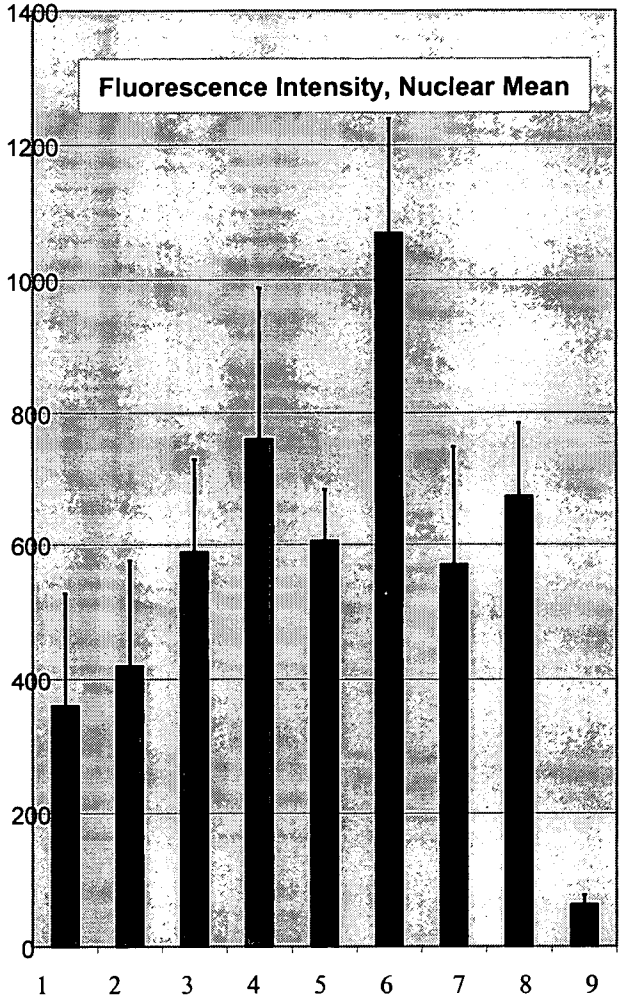


Fig. 5(A) PI3K pathway and the involvement of a novel interaction identified by PCA (hFt1/PKB)

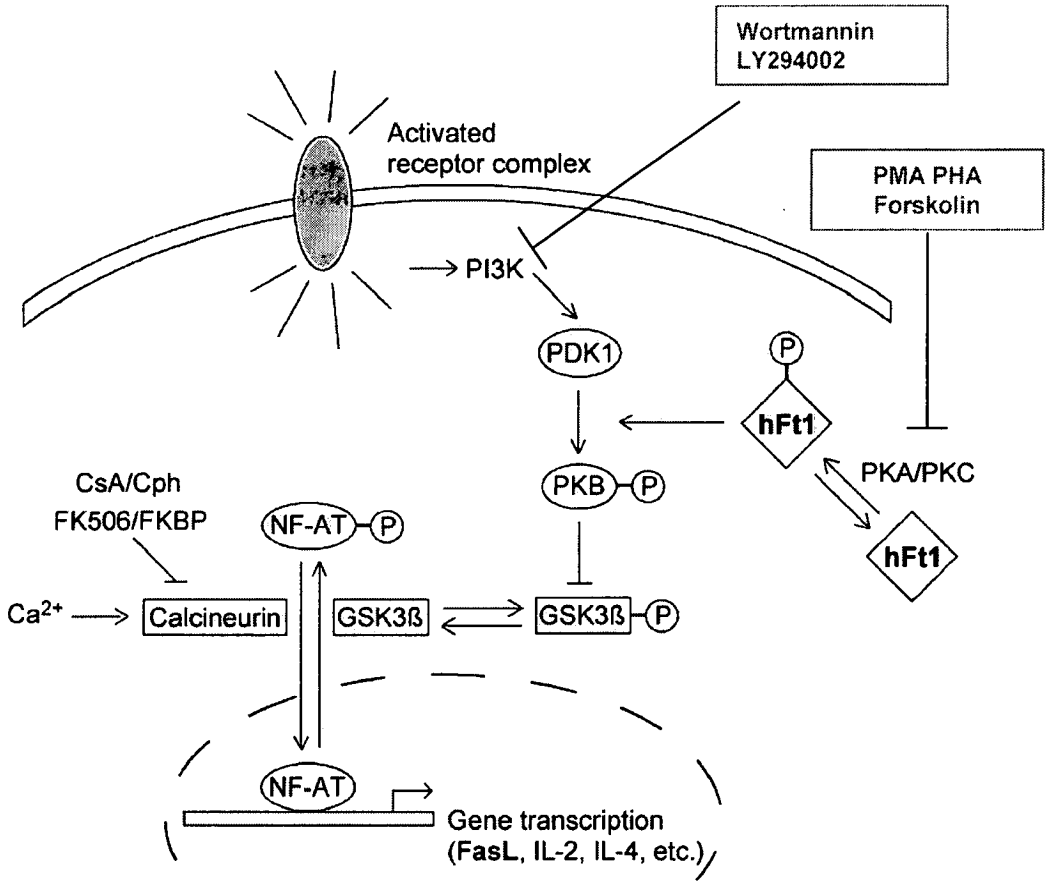


Fig. 5(B) Induction and inhibition of hFt1 complexes (GFP PCA)

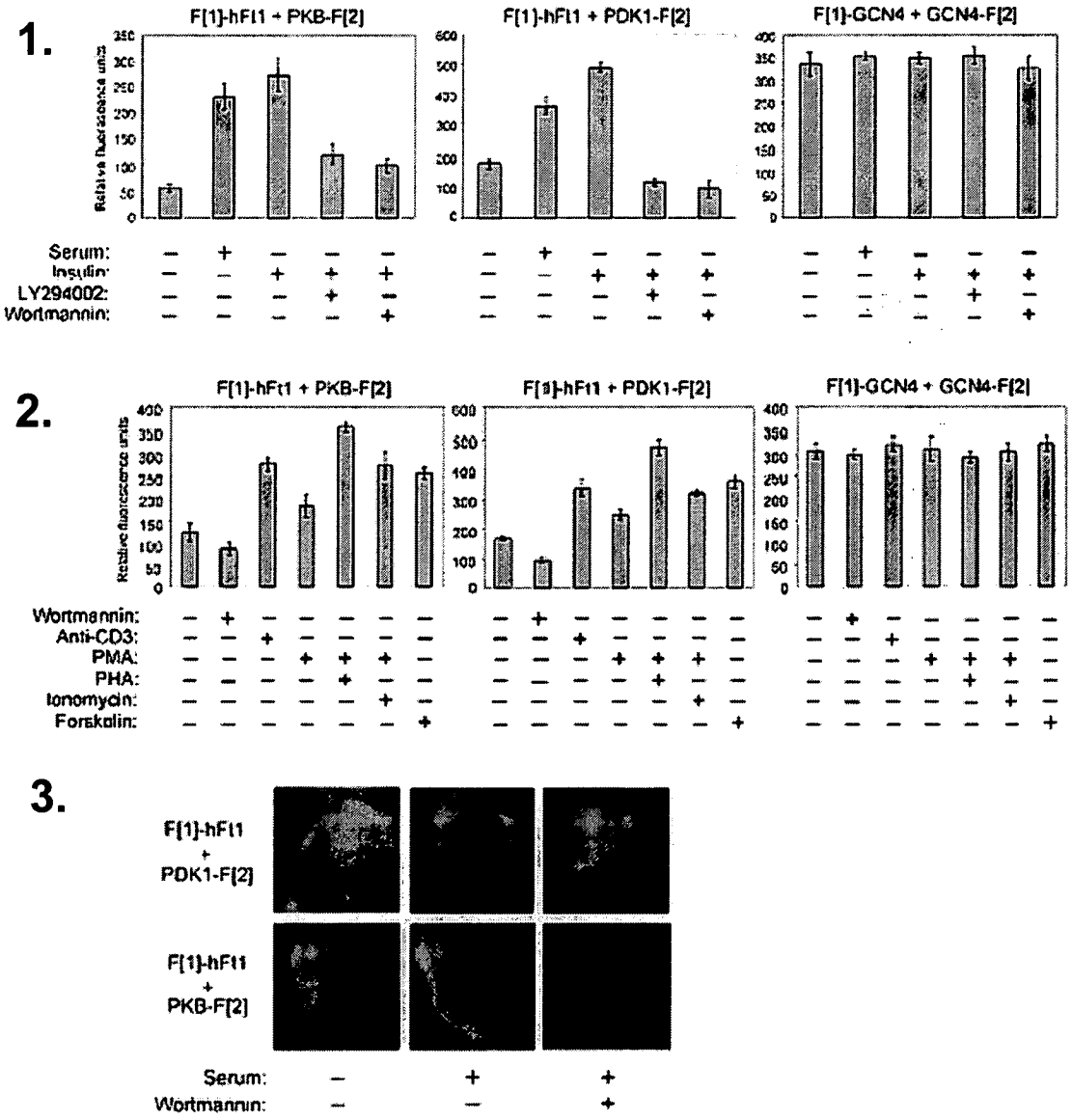
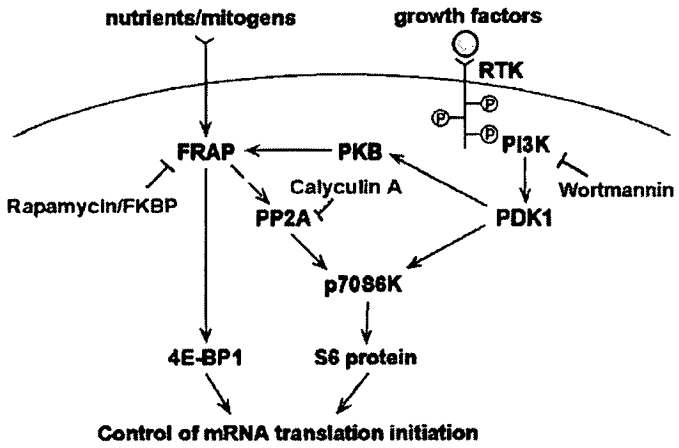
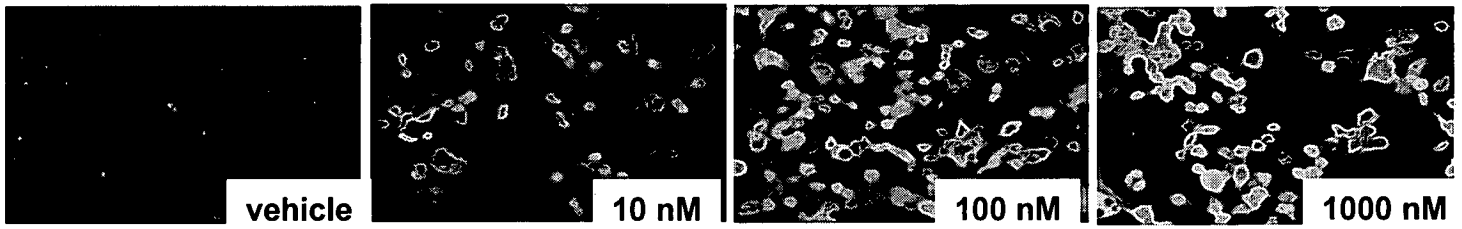


Fig. 6 A rapamycin-dependent HTS assay based on YFP PCA

A.



B.



C.

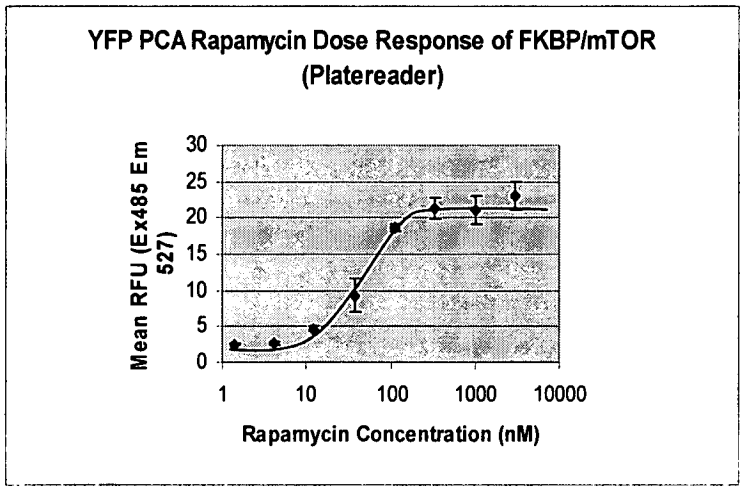


Fig. 7 Identifying interacting proteins with PCA: fluorescence spectrometry

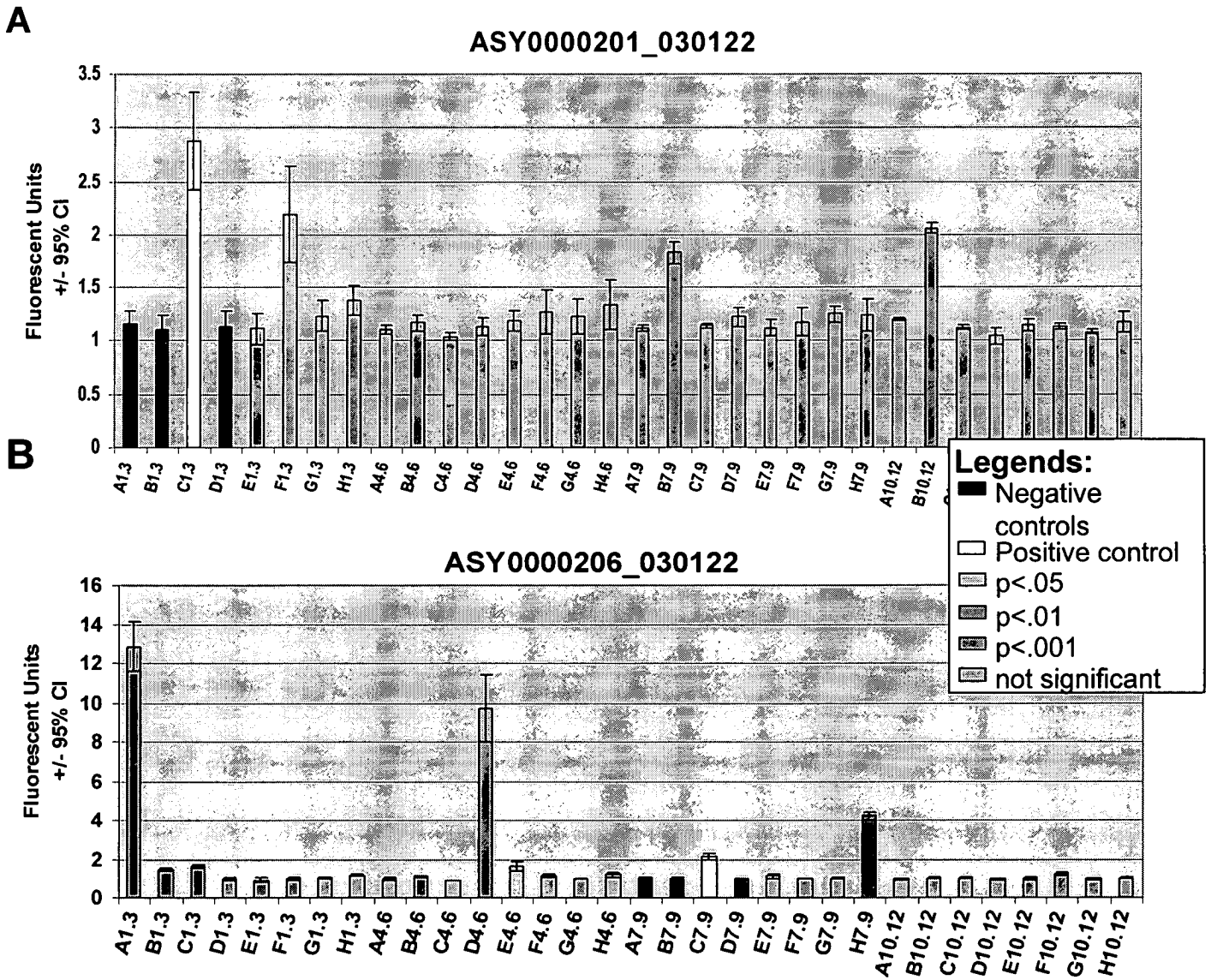


Fig 7. Identifying interacting proteins with PCA: automated microscopy

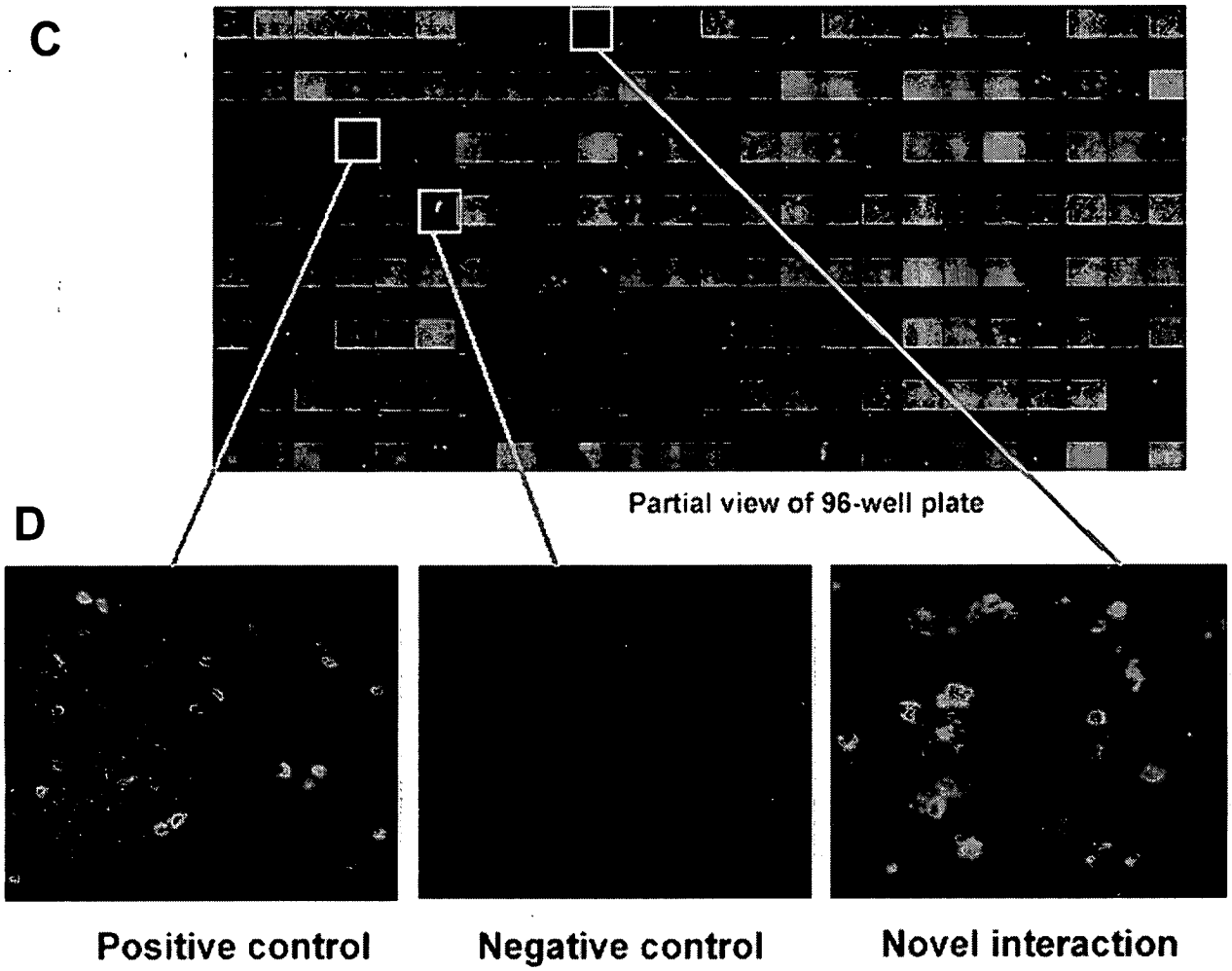


Fig. 8 TNF signaling pathway

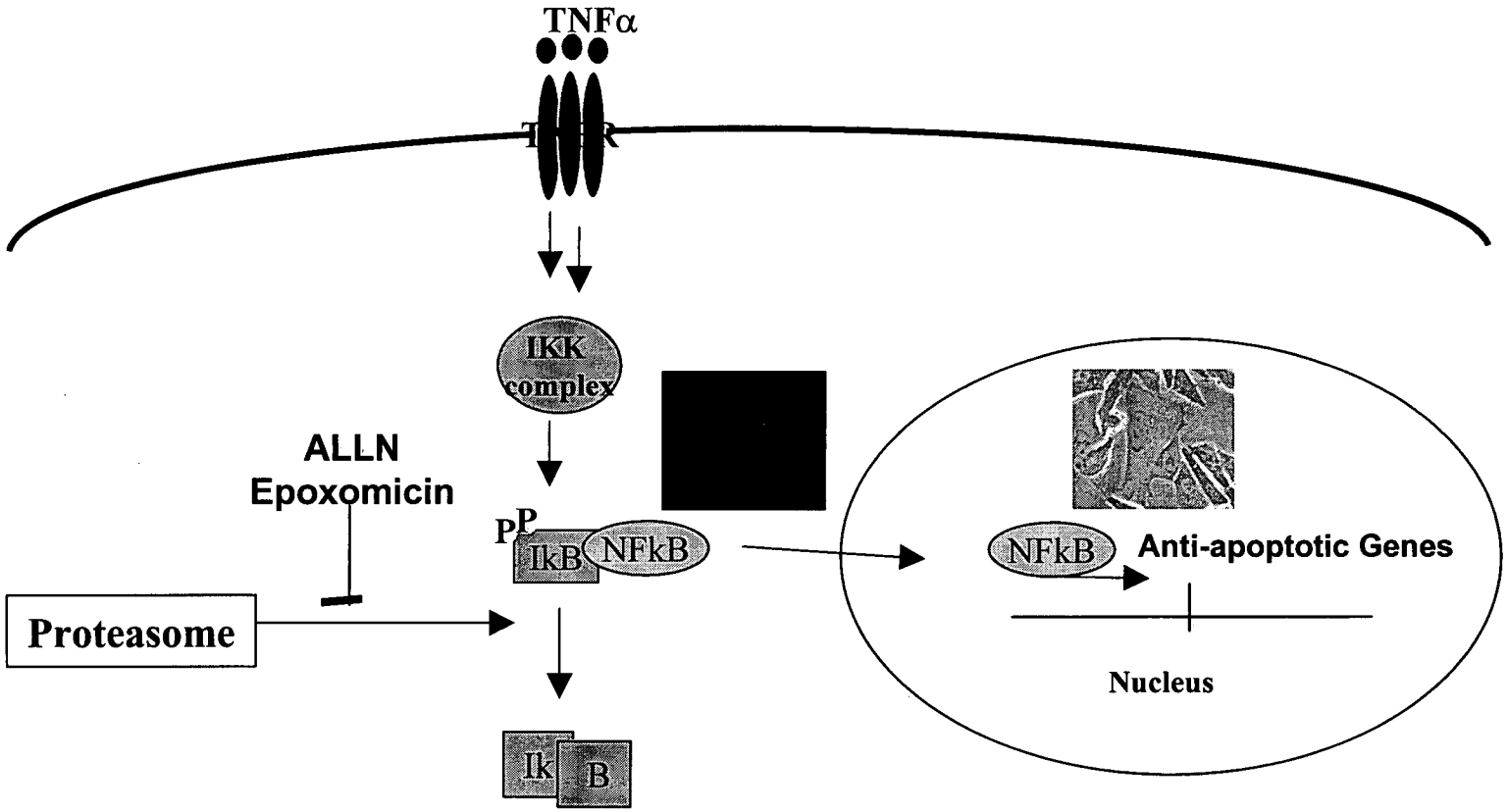


Fig. 9 Fluorescent PCAs for various steps in the TNF- and NFκB-dependent signaling pathways

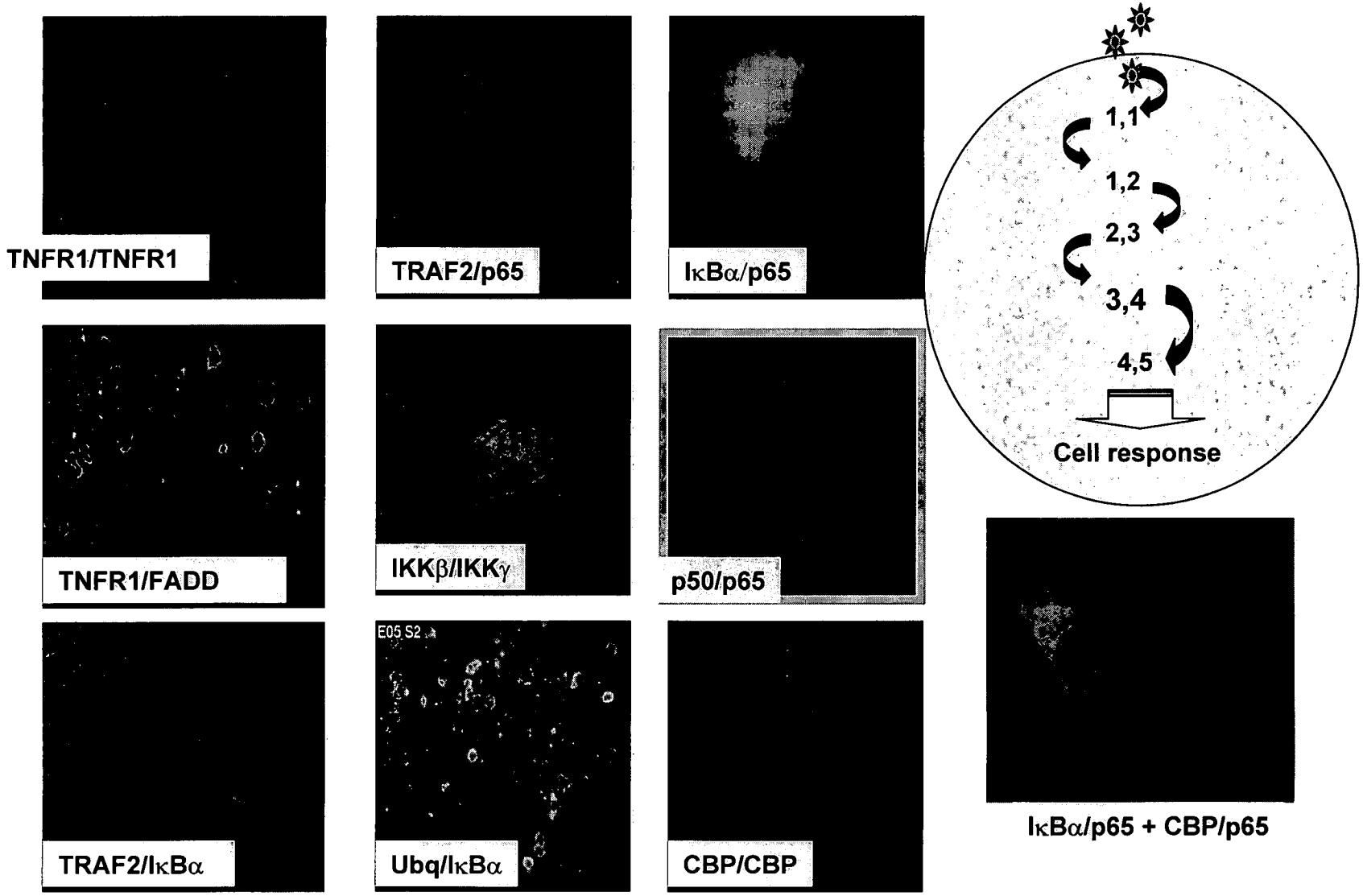


Fig. 10 TNF induction and ALLN inhibition of NFκB translocation in a transient YFP PCA

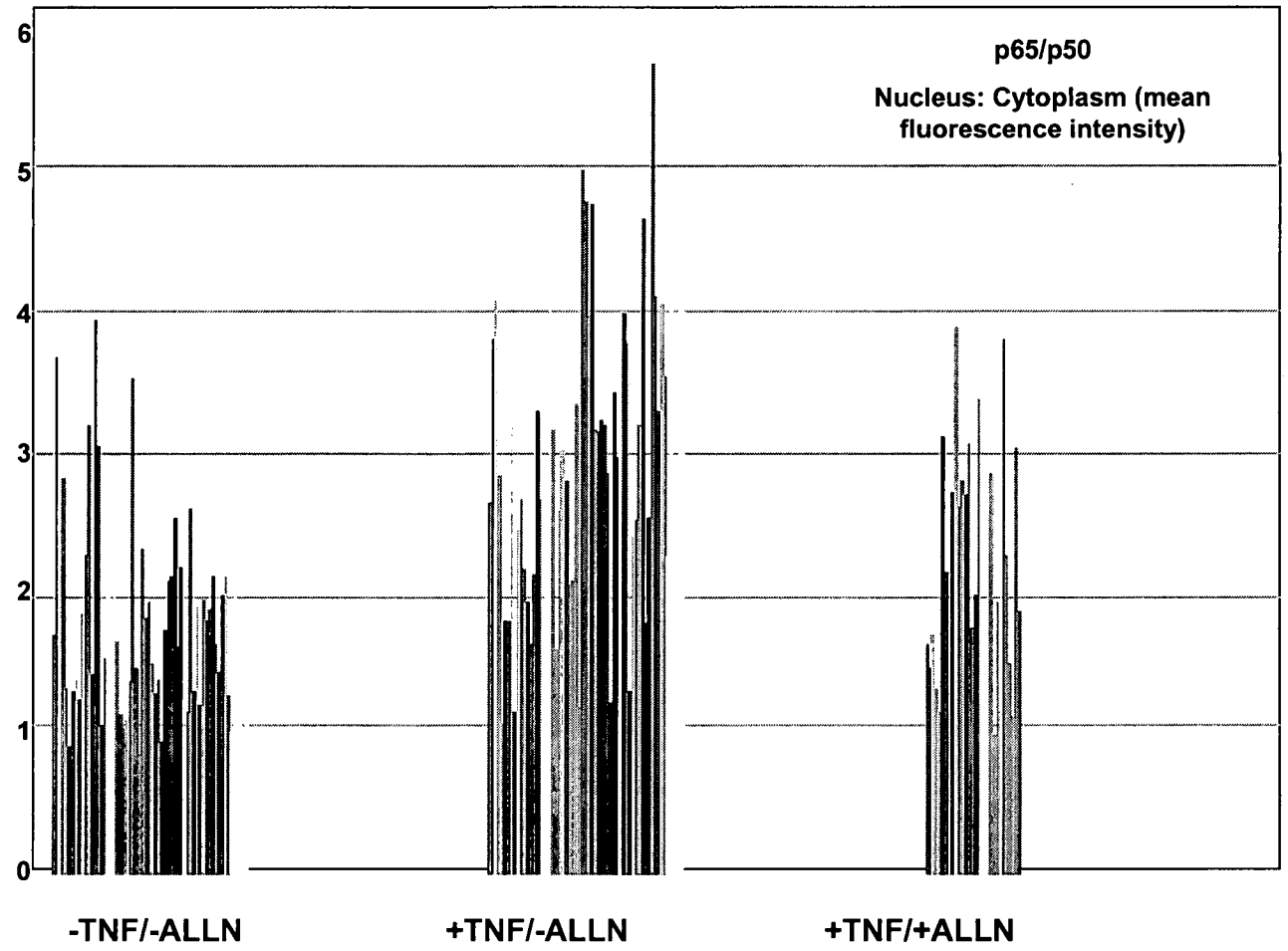
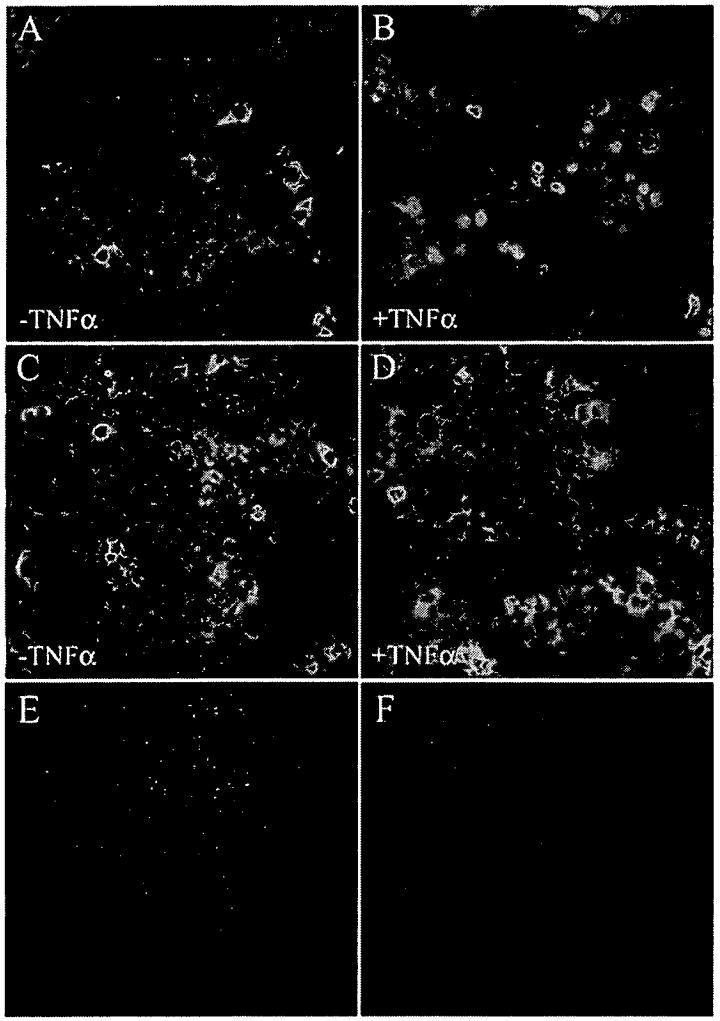


Fig. 11 Stable cell lines with PCA inside; and the absence of signal with individual gene constructs

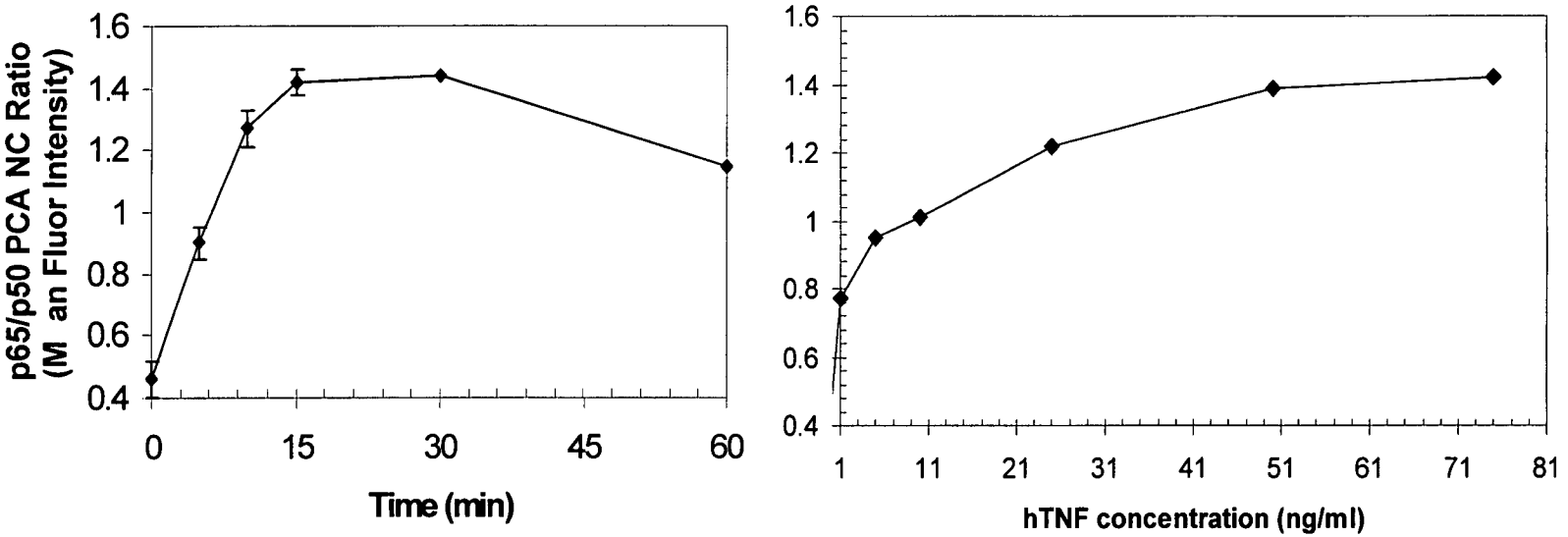


A p65/p50 PCA: - TNF
B p65/p50 PCA: +TNF

C MEK/ERK PCA: -TNF
D MEK/ERK PCA: +TNF

E YFP[2]-p65: -TNF
F YFP[2]-p65: +TNF

Fig. 12 (A) TNF induction of NFκB translocation in a stable cell line with PCA Inside



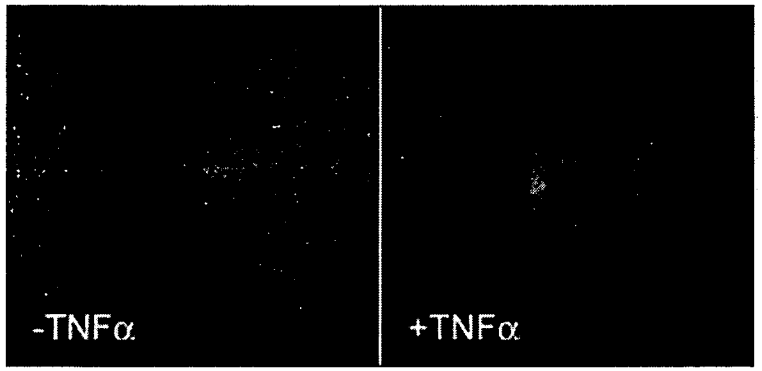


Fig. 12(B) Fluorescent high-content assay in a stable cell line showing inhibition by ALLN

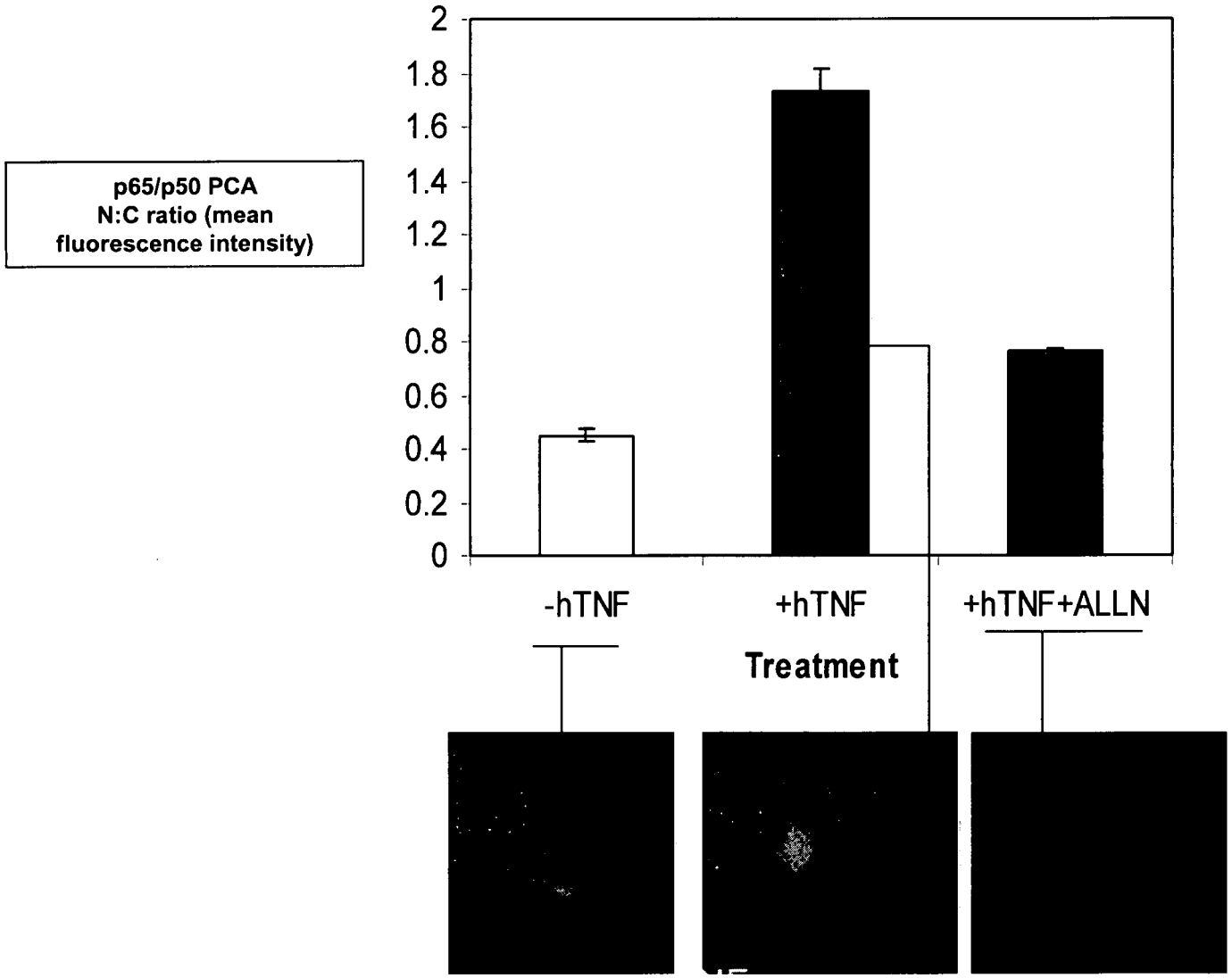


Fig. 12(C) High-content screening of a small-molecule library

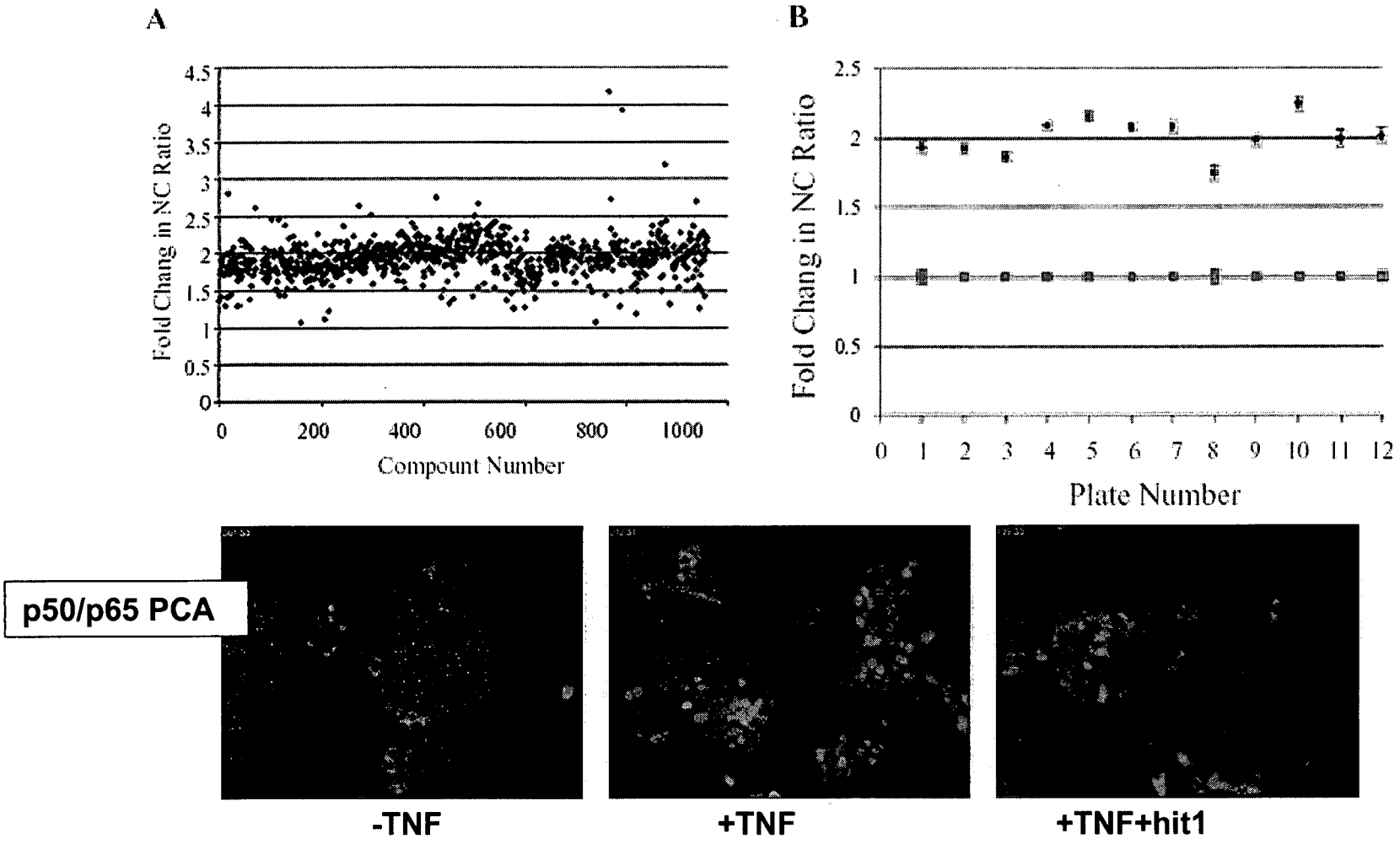


Fig. 12 (D) Dose response curve for a novel 'hit' identified by library screening

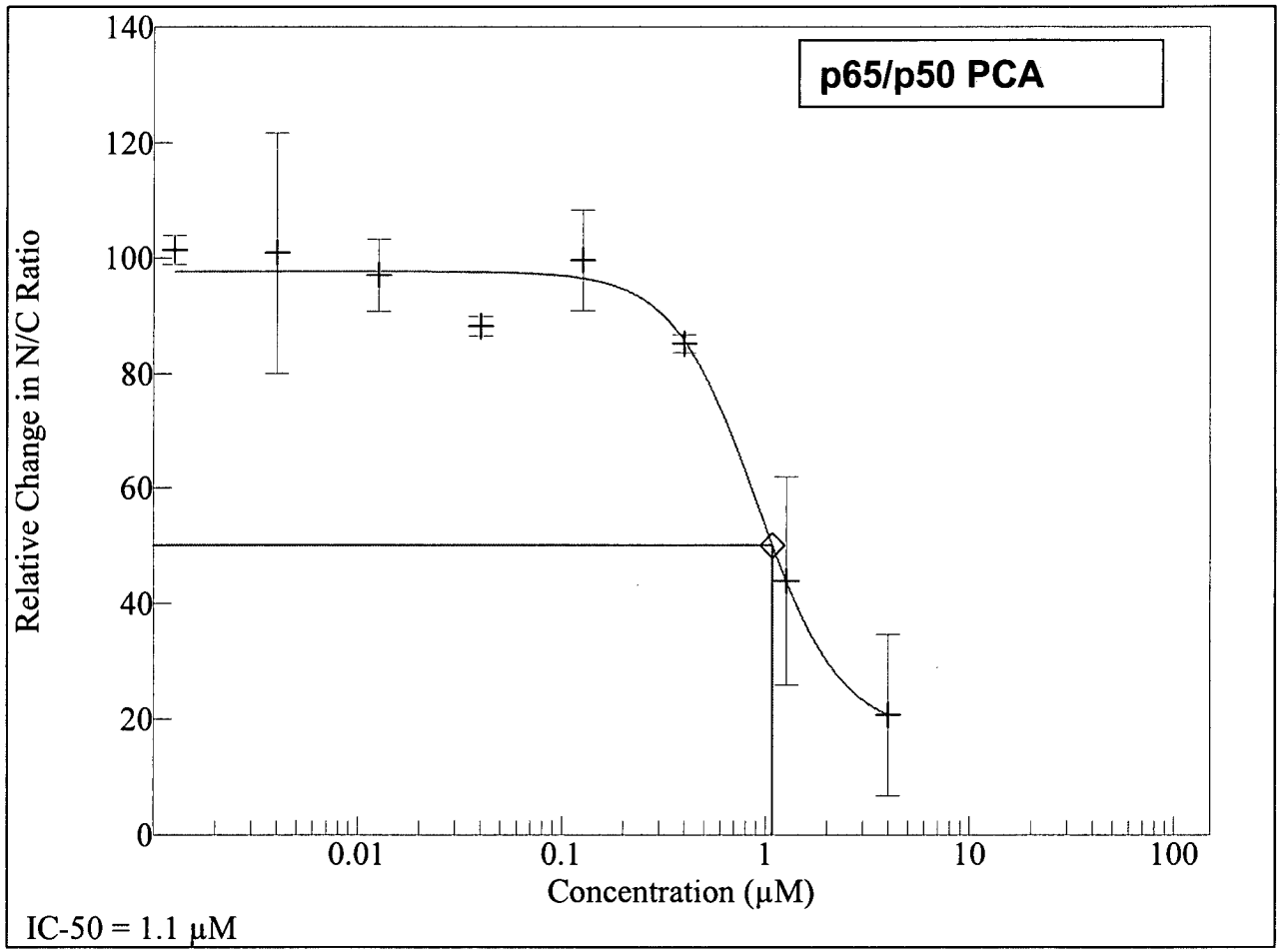
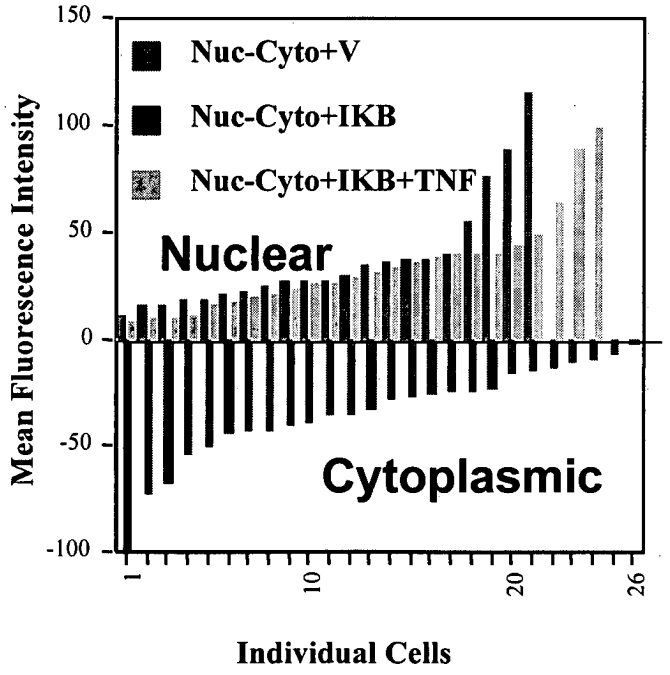
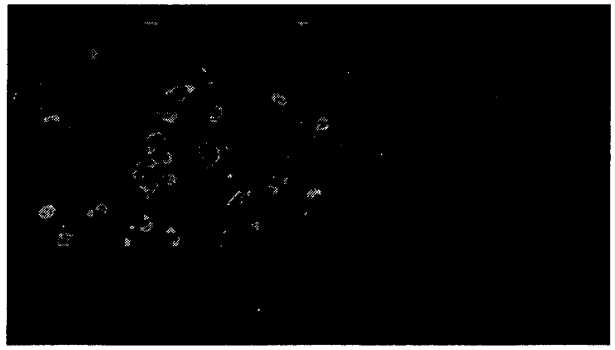


Fig. 13 (A) TNF induction of NFκB translocation (DHFR PCA in transient assays)



-TNF



+TNF

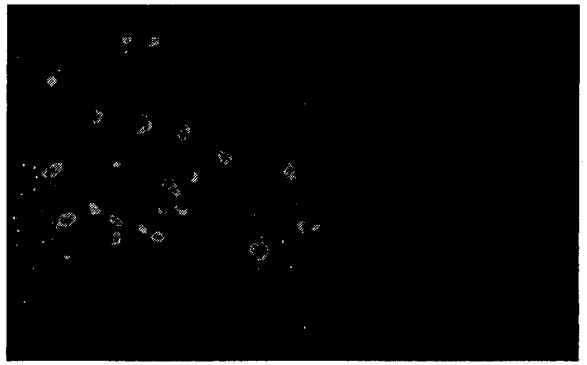


Fig. 13 (B) ALLN inhibition of NFκB translocation
(DHFR PCA in transient assays)

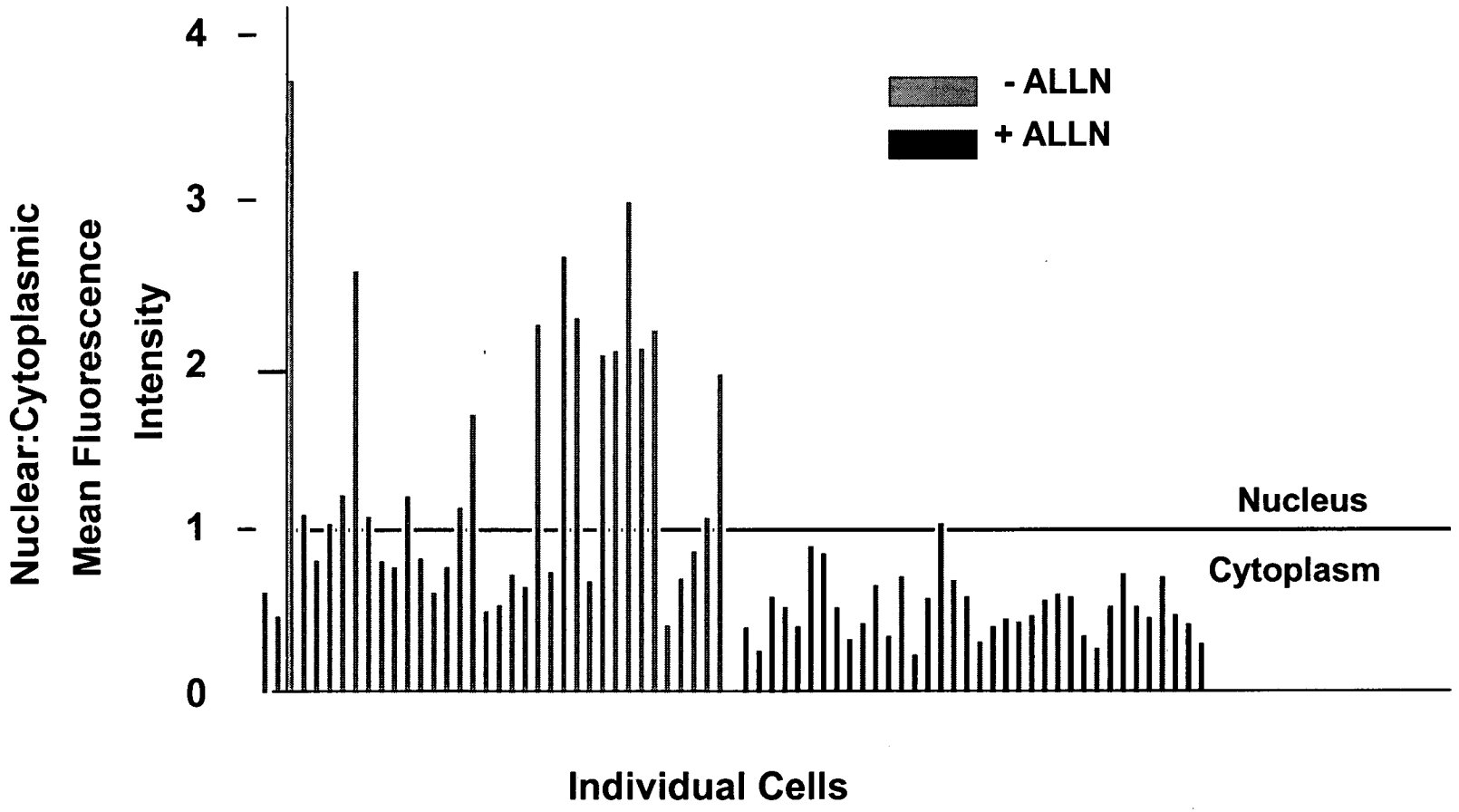
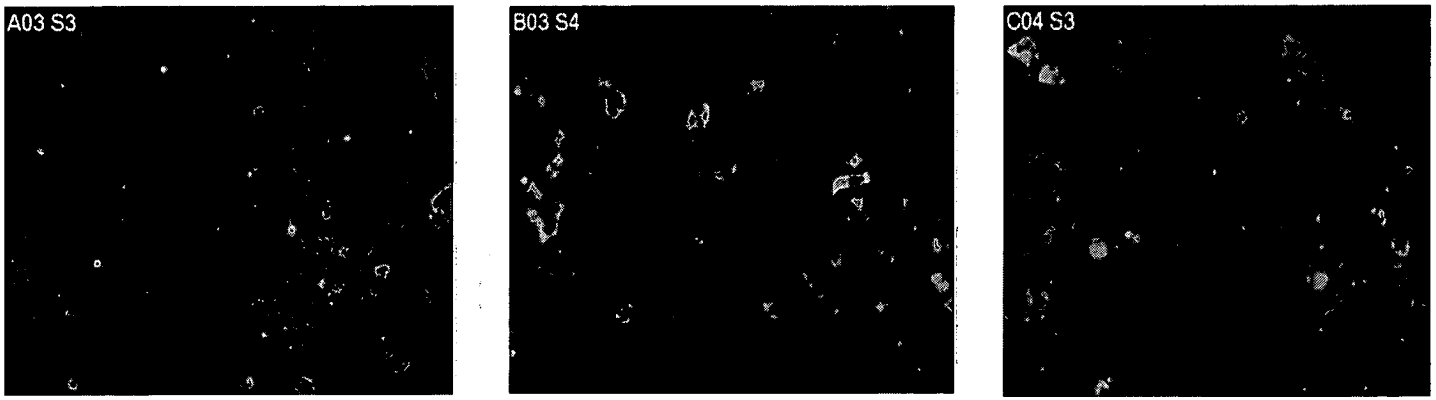


Fig. 14 Fluorescent high-throughput assay for p65/IκB in a stable cell line (PCA Inside)



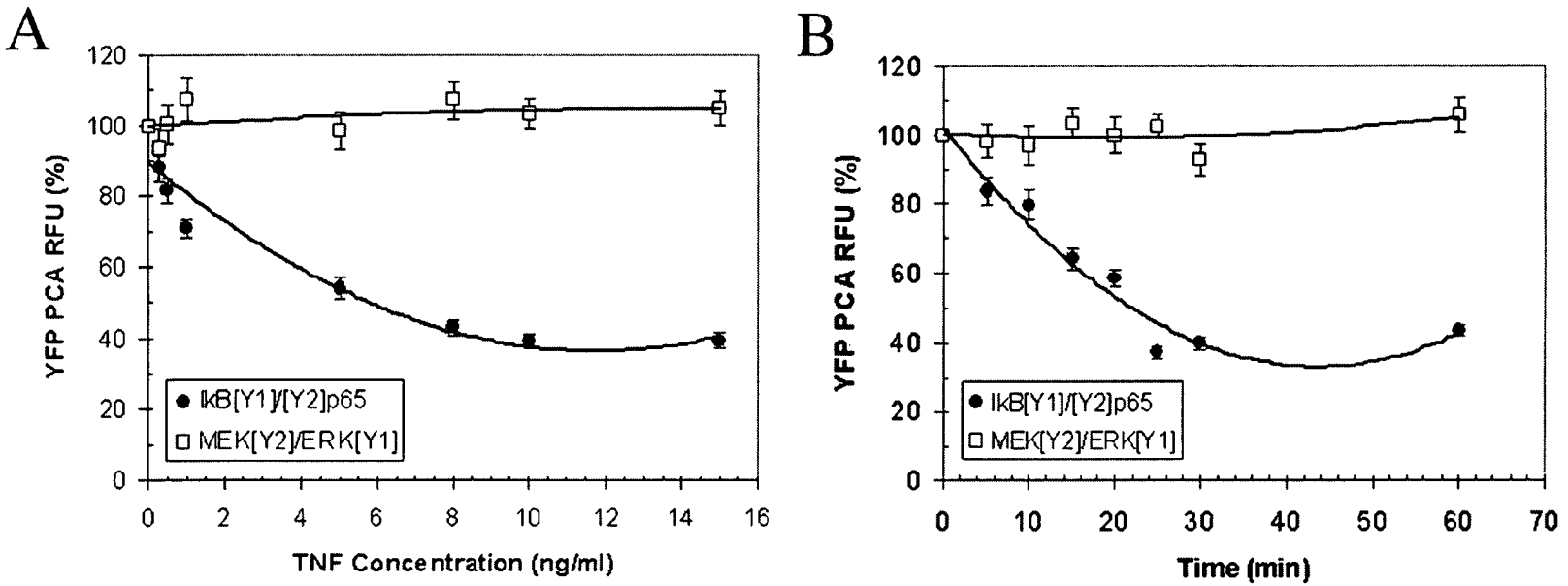


Fig. 15 Effects of TNF and ALLN on ubiquitin-protein complexes

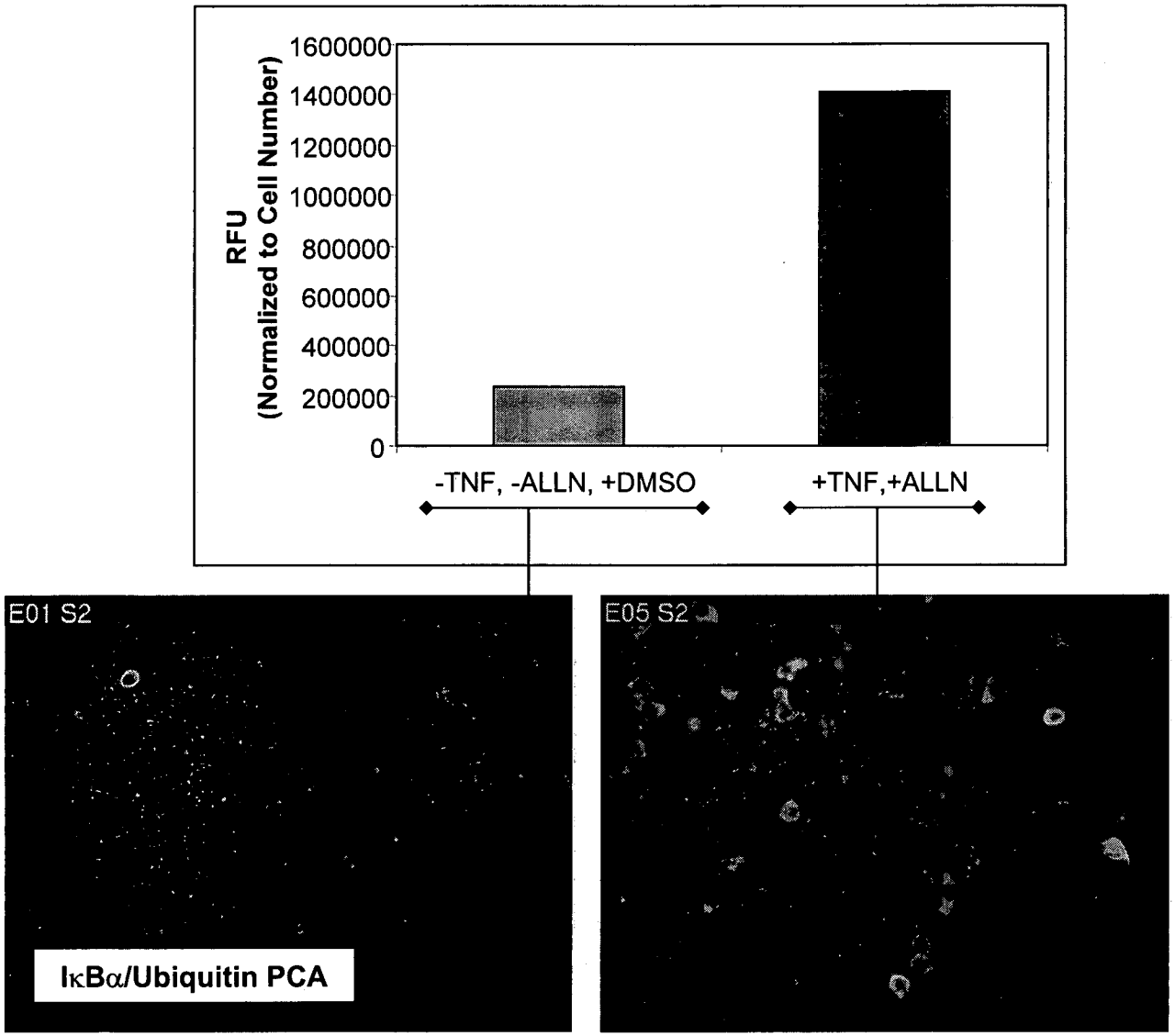


Fig. 16 Vector construction and examples

1. Select each gene (or library) of interest;
2. Select PCA fragment pair (F1, F2) suitable for the assay type;
3. Select a constitutive or inducible promoter appropriate for the cell type;
4. Subclone each gene of interest (or gene library) into one or more fragment orientations (4 possible as shown below)
5. Perform PCA with complementary (F1/ F2) pairs of constructs containing genes of interest

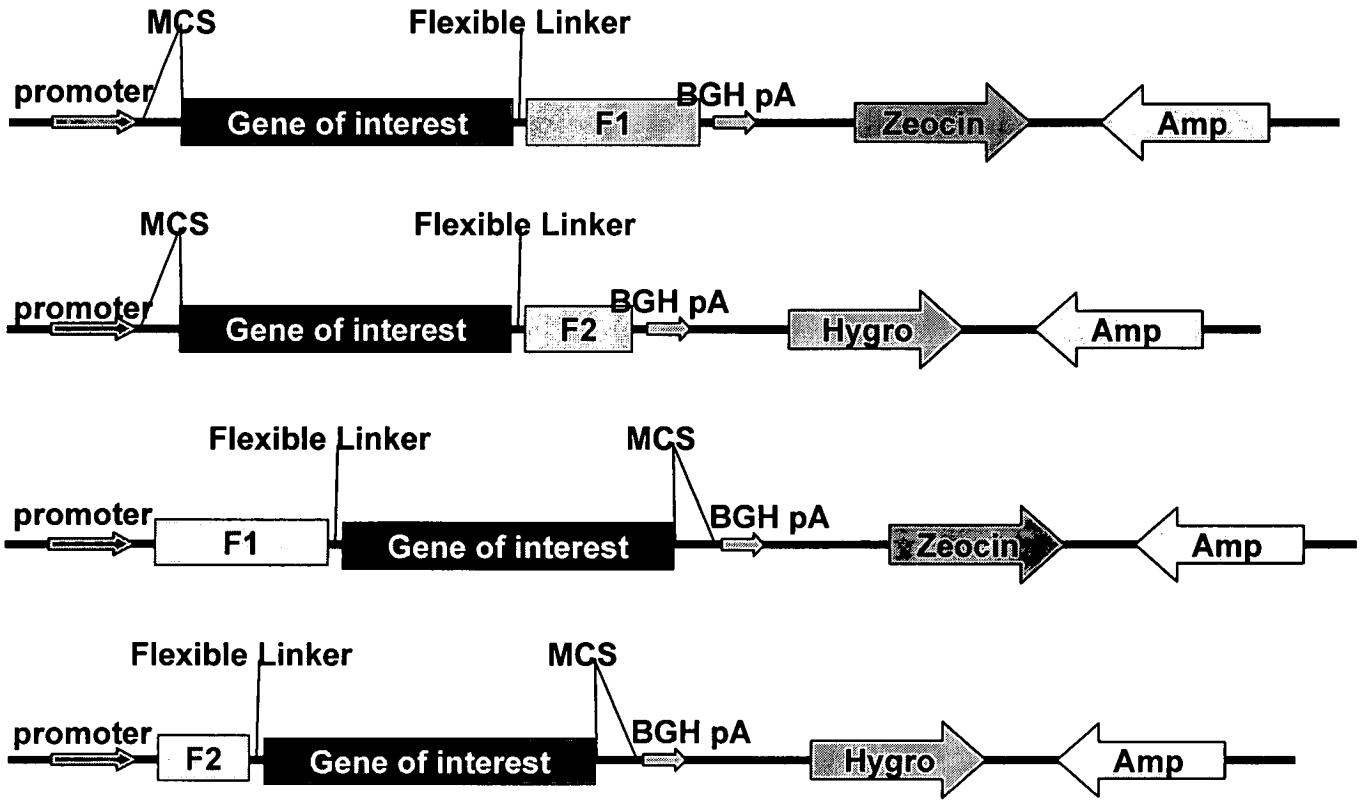


Fig. 17 Example of a Dual PCA

1. Select a survival/selection PCA (e.g. GCN4-DHFR-F[1,2]/GCN4-DHFR-F[3])
2. Select a PCA (F1, F2) suitable for HTS or HCS as described in the present invention
3. Select genes of interest (A,B) (or gene library(ies)) and subclone each gene into one or more fragments/orientations (2 possible orientations are shown below as A-F[1] and B-F[2]))
4. Apply selective pressure to cells, using growth conditions based on the survival/selection PCA (e.g. DHFR selection with MTX). Cells that survive will also co-express the A-F[1] and B-F[2] fusion proteins.
5. With the cells selected in step 4, perform a fluorescent or luminescent HTS or HCS, using the assay conditions that are specific for the PCA chosen in step 2.